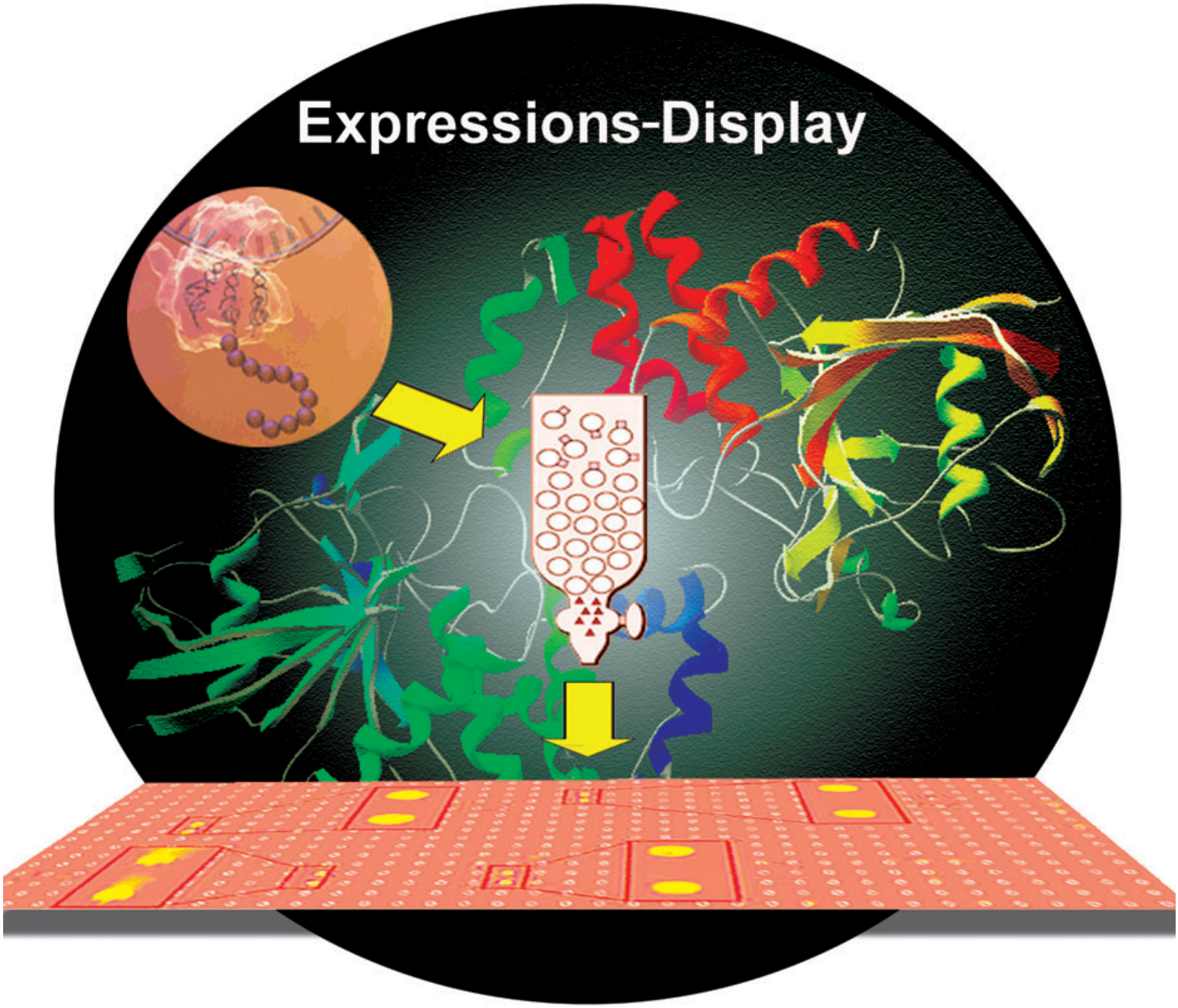
Zuschriften



Das Expressions-Display von Proteinen wird von S. Q. Yao et al. auf den folgenden Seiten vorgestellt. Die mithilfe kleiner Moleküle simultan anhand der Aktivität selektierten mRNA-gebundenen Zielenzyme werden durch Hybridisierung mit einem DNA-Mikroarray identifiziert. Im Hintergrund ist die Struktur einer Proteintyrosin-Phosphatase aus der Proteindatenbank gezeigt.

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Analytical Methods

Activity-Based High-Throughput Screening of Enzymes by Using a DNA Microarray**

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Remarkable advances in genomics have been accomplished, including the development and application of the DNA microarray technology,[1] and the recent completion of the Human Genome Project.[2] Consequently, the enormous amount of genetic information at an organism's transcriptional level is now becoming available. This situation has created the tremendous challenge to develop new techniques capable of studying the between 100000 and 1000000 functionally expressed proteins estimated in the human proteome alone.[3] Despite numerous innovations, to date, no single proteomic technique can encompass the diverse functionalities of proteins in a proteome. For example, two-dimensional gel electrophoresis (2D-GE), coupled with mass spectrometry, is primarily used to study the relative abundance, but not enzymatic activity, of proteins expressed in a biological sample.[4] Other techniques have been developed for proteome-wide analysis of protein structure,[5] localization,[6] and interactions.[7,8] One of them, the protein microarray, offers the chance to study a variety of protein activities in a large scale.[9] However, the development of this technology is largely hampered by the cost and effort needed to generate many functional proteins in sufficient purity, as well as a lack of microarray-compatible assays available to screen for different proteins, for example, enzymes spotted in a microarray.[10,11]

Enzymes are arguably the most important class of proteins, practically involved in every biological process in the cellular machinery. Many classes of enzymes, for example, proteases, kinases, and phosphatases, are linked to a variety of diseases. Traditionally, enzymes have been individually screened, identified, and characterized.[12] Recently, activity-based approaches have been reported for the study of enzymes in a proteome-wide scale.[13] However, these methods are based on electrophoretic and other chromatographic separation methods,[14,15] and require mass spectrometry for individual protein identification, which makes them less than ideal for high-throughput studies.

Protein-display technologies, which allow the generation of a large pool of encoded proteins, their display for functional selection, and rapid decoding of their structures, are particularly useful for large-scale analysis of protein activity.[16] One such technology, ribosome display, allows the in vitro expression of $>10^{13}$ proteins in a cell-free translation reaction and at the same time the proteins are "tagged" with their own coding mRNAs.[17] Although display technologies are primarily used to "evolve" proteins from a large pool of related ones (typically analogues of parental proteins generated by mutagenesis), in recent years, they have been modified to express the entire collection of proteins encoded by the complementary DNA (cDNA) in an organism.[18] Herein, we show that proteins, when expressed from a cDNA library and properly displayed (e.g. by ribosome display), are also useful for the activity-based screening of enzymes, which, when combined with the DNA microarray technology, could provide an extremely powerful strategy for the high-throughput identification and characterization of enzymes belonging in the same class(es) and, in future, other classes of non-enzyme proteins.

Our strategy, named "Expression Display", expresses proteins (in the form of ribosomal complexes with their own coding mRNAs) in a single mixture from a cDNA library (Figure 1a). Upon functional selection of enzymes belonging in the same class (e.g. PTPs) with a suitable activity-based small-molecule probe (Figure 1b), again in a single reaction mixture in the presence of other unrelated proteins, the isolated mixture (containing the desired enzymes still associated with their mRNAs) is subsequently "decoded", in a high-throughput manner, by hybridization to a DNA microarray (which contains the spatially addressable, entire genetic complement of the cDNA library) (Figure 1c,d). Noted that, although the DNA-microarray technique has been used for genome-wide screening of DNA-binding sequences of transcription factors,[19] DNA methylation,[20] and histone deacetylation,[21] as well as for the decoding of protein ligands generated from encoded combinatorial libraries,[22,23] our work is, to our knowledge, the first example where it is used for high-throughput decoding of expressed proteins (from their mRNAs).

We chose ribosome display over other display technologies (e.g. phage display and mRNA display) for protein expression and encoding, as it is in vitro-based (allowing $>10^{13}$ proteins in a library) and simple to perform (does not require DNA/RNA derivatization with puromycin[24]). To demonstrate the strategy, yeast tyrosine phosphatases were chosen as targets because the yeast proteome is well studied, and an activity-based probe targeting PTPs is available.[25]

To ensure that ribosome display expresses, isolates, and decodes functional proteins from a pool of other proteins, we constructed a model system in which streptavidin and EGFP (enhanced green fluorescent protein; 100-fold excess) were displayed. Upon enrichment with biotin, the mRNA corresponding to streptavidin was preferentially isolated and confirmed (see Supporting Information). We tested whether

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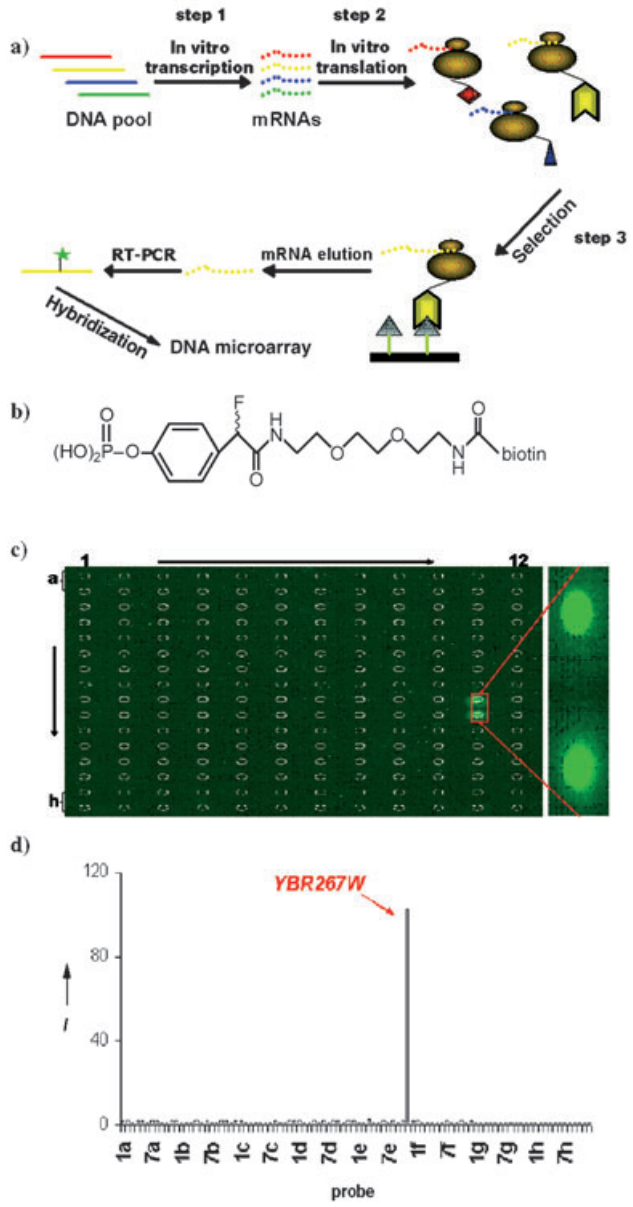

Figure 1. a) Schematic representation of expression display; RT = reverse transcription, PCR = polymerase chain reaction, see text for full details. b) The structure of the activity-based probe specific for protein tyrosine phosphatases (PTPs). c) The hybridization of Cy3-labeled reverse transcripts from in vitro selection onto a "decoding" DNA microarray containing 96 yeast ORFs (in duplicate). The spotting pattern is highlighted by white circles. The numbering of spots, their corresponding genes, and relative fluorescence intensities are in the Supporting Information. The positive spots, representing *YBR267W* (in duplicate), are highlighted in the enlargement. d) Relative fluorescence intensities (*I*) of spots from the DNA microarray after hybridization. The intensity values of replicate spots were averaged.

a ribosome-displayed, mRNA-tethered PTP could be isolated from other proteins with the activity-based probe (shown in Figure 1b), and subsequently identified by a microarray spotted with individual "decoding" DNAs (that is, complementary to the mRNAs tethered to the protein library). We isolated plasmids containing 96 different yeast open-reading frames (ORFs), one of which is *YBR267W*, encoding a known PTP (i.e. positive control). To ensure that all genes were equally represented in the DNA library, all yeast ORFs were individually amplified with the same pair of primers. Subsequently, multistep reassembly PCR was performed to introduce suitable spacers/linkers, as well as the T7 promoter/terminator and other components essential for ribosome display. Removal of the stop codon in the genes is optional, but it was carried out throughout our experiments to maximize the yield of displayed proteins.[17] Upon pooling into a single mixture, the resulting DNA library was transcribed and translated to generate a mixture of ribosome-displayed proteins from the 96 genes (Step 1 in Figure 1a). Subsequently, streptavidin magnetic beads immobilized with a biotinylated activity-based probe (Figure 1b) were used to isolate any PTP present in the mixture (Step 2). The probe reacts irreversibly with PTPs (as well as some alkaline phosphatases;[25]) in a highly specific, activity-dependent manner. The washing steps were optimized to remove any residual protein–ribosome-mRNA complex from the beads without causing dissociation of the complex itself. The mRNAs from the probe-bound complexes were subsequently eluted and purified. To decode (Step 3), the isolated mRNAs were reversely transcribed to generate the corresponding fluorescently labeled cDNAs which were then hybridized to the "decoding" DNA microarray, and identified by virtue of the location of fluorescent spots on the array (Figure 1c,d). The DNA microarray (constructed in house), contains individual cDNA from all 96 yeast genes. Results showed that the only fluorescent spot on the microarray was that of *YBR267W* (i.e. the positive hit). A control hybridization experiment with a Cy3-labeled PCR product obtained using mRNAs before ribosome display/selection showed a fairly homogeneous distribution of fluorescent spots throughout the array (data not shown). In a separate experiment, isolated mRNAs (after Step 2) were reversely transcribed, amplified by PCR, and cloned into pCR2.1-TOPO vector (Invitrogen), subsequent DNA sequencing showed: 10 out of 10 randomly chosen clones corresponded to the *YBR267W* gene. When sodium orthovanadate, a potent tyrosine phosphatase inhibitor, was added to the incubation mixture during expression display (i.e. at Step 2), the selection of *YBR267W* was abolished (see Supporting Information). Together, all these lines of evidence validated our strategy, that is, enzymes expressed from a cDNA library using ribosome display could be preferentially isolated on the basis of their enzymatic activity (by the aid of a suitable activity-based probe) and subsequently identified, in high-throughout, by hybridization to a DNA microarray.

To assess whether expression display could be used for high-throughput proteomics mass screening of enzymes in a class-specific manner, we applied our strategy to a yeast cDNA library containing 384 different yeast ORFs, including multiple previously characterized PTPs (four in total; including *YBR267W*, *YDL230W*, *YFR028C*, and *YPR073C*), non-PTP phosphatases (see below), other classes of enzymes (proteases, kinases, oxidoreductases), and non-enzyme proteins. Once again, all genes were individually PCR-amplified and assembled before pooling to ensure equal representation in the library. Upon in vitro transcription and translation, the mixture containing the ribosome-display proteins was subject

to activity-based enrichment, in a single reaction, using the same small-molecule probe. The isolated mixture was reversely transcribed, amplified with Cy3-labeled dNTPs, and hybridized to a "decoding" DNA microarray (containing 384 cDNAs) for parallel identification of PTPs (Figure 2a): upon subtraction of background fluorescence, the only significantly fluorescent spots identified from the microarray were from those genes encoding the four PTPs (Figure 2b). The identities of these genes were independently confirmed by DNA sequencing, as described above. The enzymatic activities of the corresponding proteins were further confirmed by successful labeling of the individually purified proteins with the probe in a gel-based experiment, as well as inhibition experiments with sodium orthovanadate (see Supporting Information). Noticeably, the relative intensity of the fluorescent spots in Figure 2a,b (with 384 genes) was considerably weaker than those in Figure 1c,d (with 96 genes), presumably a result of the decreased expression of each protein from a larger cDNA library (theoretically fourfold less expression from the same translation mixture, assuming equal expression among different genes).

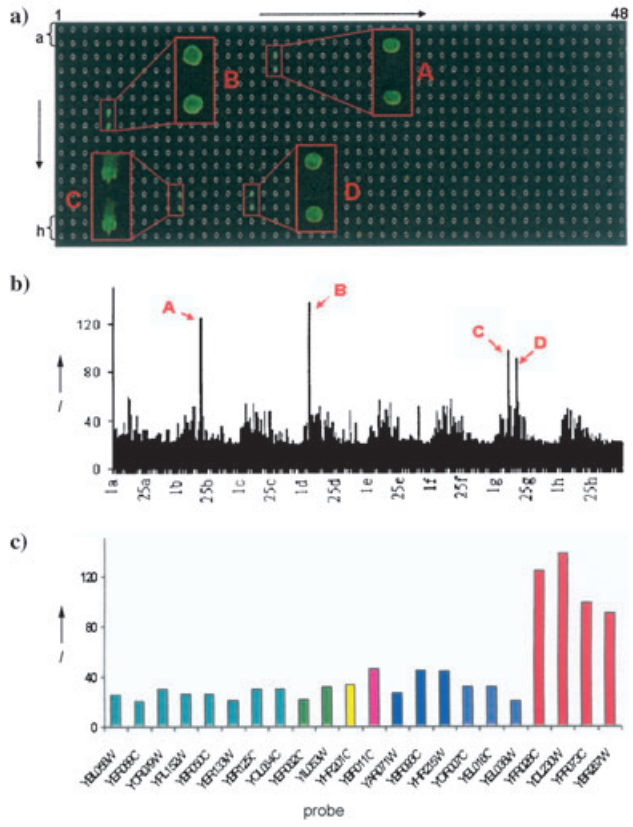

Figure 2. Rapid identification of multiple yeast PTPs using expression display. a) The hybridization of Cy3-labeled reverse transcripts from in vitro selection onto a "decoding" DNA microarray containing 384 yeast ORFs (in duplicate). The spotting pattern is highlighted by white circles. The numbering of spots, their corresponding genes, and relative fluorescence intensities are given in the Supporting Information. The positive spots are highlighted in the enlargements. Four positives were identified as A: *YFR028C*, B: *YDL230W*, C: *YPR073C*, and D: *YBR267W*, corresponding to the four positive PTPs in the cDNA library. b) Relative fluorescence intensities (*I*) of spots from the DNA microarray after hybridization. The intensity values of replicate spots were averaged. The four positive PTPs are labeled (A–D). c) Comparison of relative fluorescence intensities of the four PTPs (red) to those of other phosphatases in the cDNA library, including eight Ser/Thr phosphatases (cyan), two DL-glycerol phosphatases (green), one exopolyphosphatase (yellow), one inorganic pyrophosphatase (pink), three acid phosphatases (dark blue), and three phosphatase homologues (light blue).

The small-molecule probe used in the above experiments is highly specific towards PTPs.[25] To assess whether proteins identified from expression display retain a similar fidelity as defined by the probe (in the specific targeting of PTPs), we included in the reaction mixture other non-PTP phosphatases. As shown in Figure 2c, within a single round of selection, only the four PTPs emerged as positive hits, as their fluorescence intensities were clearly enriched over those of the eighteen non-PTP phosphatases proteins, as well as the rest of proteins in the library. Together, with the availability of new activity-based probes,[26–28] our strategy may be readily modified to accommodate different classes of enzymes which confer either highly specific or broad-based specificities, thus providing a novel and general means in high-throughput enzymology.

In conclusion, we have demonstrated that expression display could be used for high-throughput screening and identification of proteins on the basis of their enzymatic activities: with an activity-based probe, we have shown, for the first time, multiple enzymes in the same class (e.g. PTPs), when expressed (from a cDNA library together with other proteins) in a single mixture as ribosome-displayed complexes, could be isolated, and subsequently identified, in high throughput, using DNA microarray as the "decoding" platform (this is also the first example of protein decoding). In our experiments, all genes were individually cloned before pooling into a single cDNA library to preserve their diversity and equal distribution, and to unambiguously validate our strategy. Other cDNA libraries, either commercially available or constructed using standard cloning techniques, should be equally amendable. Our strategy is unique in a number of ways when compared with existing proteomic techniques: 1) it differs from the electrophoretic/chromatographic protein profiling methods (including activity-based methods;[13–15,25–28]) by allowing high-throughput identifications of enzymes without the need for mass spectrometry; 2) by avoiding the need for parallel cloning, expression, purification, and characterization of proteins (as in the case reported in ref. [12]), it could (in principle) routinely express, screen, and identify thousands (if not millions/billions) of proteins, all in a single reaction mixture; 3) by utilizing RT-PCR for the amplification of isolated mRNAs and subsequently decoding by hybridization, it should in principle provide a very low detection limit for target proteins. What remains unaddressed from this study, is the maximum number of different proteins allowed in the strategy? Clearly, from our results, shown in Figure 2, with a single round of protein selection (e.g. no amplification of the input protein amount), the fluorescence intensity of positive hits (from DNA microarray screening) decreased concomitantly with the increasing cDNA library size. However, with the easy adaptation of multiple rounds of protein enrichment (as in the case of ribosome display, and

other display technologies), the capacity of our strategy may be greatly expanded, thus making it possible in future to "display" and screen all proteins, including low-abundant ones, present in a proteome (> 1 000 000 proteins).

Experimental Section

DNA constructs: Details of the cloning are described in Supporting Information. Briefly, yeast ORFs were amplified from plasmids of ExClones (Invitrogen, USA) with the following primers: Exclone-YF (5′-GCGGCGGCCATATGGAATTCCAGCTGACCACC-3′) and Exclone-YR (5′-GGCGGCTGCTCTTCCGCATCCCCGGGAA-TTGCCATGCCA-3′), which removed the stop codon from each ORF, and presumably maximized the yield of ribosome-displayed proteins.[17] However, it was shown previously that, without removal of the stop codon, ribosome display also worked.[29] A spacer was amplified from amino acids 211–299 of gene *III* of filamentous phage M13mp19 with the primers Y-spacer-F (5′-GGGGATGCGGAA-GAGCAGCCGCCCCCTCAACCTCCTGTCAAT-3′) and Spacer-R (5′-CCGCACACCAGTAAGGTGTGCGGTATCACCAG-TAGCACC-3′), and subsequently ligated to the amplified yeast ORFs: both the ORF and the spacer were digested by Sap I (NEB, USA) at 37 °C for 2 h and subsequently ligated with T4 ligase (NEB) overnight at 16 °C. The ligation products were amplified first by two primers, Y-SDA (5′-AGACCACAACGGTTTCCCTCTAGAAA-TAATTTTGTTTAACTTTAAGAAGGAGATATATCCATG-GAATTCCAGCTGACCACC-3′) and Spacer-R, followed by amplification with primers Y-T7B (5′-ATACGAAATTAATACGACT-CACTATAGGGAGACCACAACGG-3′) and Spacer-R. All final constructs were re-amplified with the primers Y-T7B and Spacer-R, if necessary. Streptavidin and EGFP genes were constructed similarly (Supporting Information).

In vitro transcription and translation: Depending on the experiments, different DNA constructs were pooled proportionally to obtain a master mixture. 1–6 μg of the mixture was transcribed with the RiboMAX large-scale RNA production system T7 (Promega) for 4 h at 37 °C. The mRNAs were purified by an RNeasy mini kit (Qiagen). The resulting transcripts were translated in vitro with an *E. coli* translation system (Roche) in the presence of magnesium acetate (10 mM), anti-ssrA (5.6 μM; 5′-TTAAGCTGCTAAAGCG-TAGTTTTCGTCGTTTGCGACTA-3′) and rRNasin (0.5 μL 40 U μL^{-1}; Promega). The translation was performed in a 35 μL reaction for 7 min at 37 °C, before stopping by immediately transferring the product into an ice-cold binding buffer (220 μL) containing Tris-acetate (50 mM; pH 7.5 Tris = 2-amino-2-(hydroxymethyl)-1,3-propanediol), NaCl (150 mM), magnesium acetate (50 mM), Tween 20 (0.1 % (v/v), bovine serum albumin (0.1 mg mL^{-1}) and heparin (2 mg mL^{-1}).

In vitro selection: The small-molecule probe[25] (50 μL, 300 μM) was incubated with Streptavidin MagneSphere paramagnetic particles (50 μL; Promega) for 30 min at room temperature with gentle shaking, followed by washing with 1 × PBS (three times; PBS = phosphate buffered saline) to remove any excessive free probe. Subsequently, the above translation mixture, premixed with the binding buffer, was incubated with the beads for 2 h on ice. For the control selection with streptavidin/EGFP, immobilized iminobiotin agarose (20 μL; Pierce) were added to translation mixture and incubated for 1 h on ice. After incubation, the beads (agarose for streptavidin reaction) were washed four times with a washing buffer containing Tris-acetate (50 mM; pH 7.5), NaCl (150 mM), magnesium acetate (50 mM) and Tween 20 (0.1 % (v/v)). The washing conditions were optimized to remove residual, displayed proteins without breaking the ribosome complexes. Subsequently, mRNAs were released from the ribosome ternary complexes at room temperature with an elution buffer containing Tris-acetate (50 mM; pH 7.5), NaCl (150 mM) and EDTA (20 mM; EDTA = N,N′-(1,2-ethanediyl)bis[(N-carboxymethyl)-glycine]). The eluted mRNAs were purified by RNeasy mini kit (Qiagen).

RT-PCR: Reverse transcription was performed using AMV reverse transcriptase (Promega) with the primer, Spacer-R, according to the supplier's recommendation. PCR was performed using Taq polymerase (Promega) in the presence of 5 % (v/v) dimethyl sulfoxide (5 min at 95 °C, followed by 25 cycles of 30 s at 95 °C, 30 s at 50 °C and 2.5 min at 72 °C, with a final 10 min of extension at 72 °C). PCR products were analyzed by agarose gel electrophoresis. For fluorescent labeling of RT-PCR product, Cy3-dCTP (Amersham Biosciences) was incorporated into PCR products according to the supplier's recommendation. The fluorescent DNAs were subsequently used as probes for DNA microarray hybridization. For direct cloning and DNA sequencing, see the Supporting Information

DNA microarray: All DNA templates used for spotting the "decoding" DNA microarrays were amplified, in 96-well formats with primers Y-T7B and Spacer-R, from plasmids containing the corresponding yeast ORFs from Exclones. DNA spotting and hybridization were performed as described elsewhere,[30] with the following modifications: yeast ORFs were spotted in duplicate onto 75 × 25 mm polylysine-coated glass slides using a CHIPWRITER arrayer (Virtek), followed by incubation in a humid chamber overnight at room temperature. After rehydration, blocking, and denaturing, the slides were dried and ready for DNA hybridization. The probes were the Cy3-labeled reverse transcripts from earlier in vitro selection experiments. The hybridization chambers were kept in a humid environment at 60 °C for 2–4 h or overnight. After washing, the slides were analyzed by ArrayWoRx scanner (Applied Precision). The values of signal intensity of duplicate spots were averaged for each ORF (Supporting Information).

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[1] D. J. Lockhart, E. A. Winzeler, *Nature* **2000**, *405*, 827 – 836.
[2] J. C. Venter, *Science* **2001**, *291*, 1304 – 1351.
[3] S. Fields, *Science* **2001**, *291*, 1221 – 1224.
[4] R. Aebersold, M. Mann, *Nature* **2003**, *422*, 198 – 207.
[5] S. K. Burley, *Nat. Struct. Biol.* **2000**, *7*, 932 – 934.
[6] W. K. Huh, J. V. Falvo, L. C. Gerke, A. S. Carroll, R. W. Howson, J. S. Weissman, E. K. O'Shea, *Nature* **2003**, *425*, 686 – 691.
[7] A. C. Gavin, M. Bosche, R. Krause, P. Grandi, M. Marzioch, A. Bauer, J. Schultz, J. M. Rick, A. M. Michon, C. M. Cruciat, M. Remor, C. Hofert, M. Schelder, M. Brajenovic, H. Ruffner, A. Merino, K. Klein, M. Hudak, D. Dickson, T. Rudi, V. Gnau, A. Bauch, S. Bastuck, B. Huhse, C. Leutwein, M. A. Heurtier, R. R. Copley, A. Edelmann, E. Querfurth, V. Rybin, G. Drewes, M. Raida, T. Bouwmeester, P. Bork, B. Seraphin, B. Kuster, G. Neubauer, G. Superti-Furga, *Nature* **2002**, *415*, 141 – 147.
[8] Y. Ho, A. Gruhler, A. Heilbut, G. D. Bader, L. Moore, S. L. Adams, A. Millar, P. Taylor, K. Bennett, K. Boutilier, L. Y. Yang, C. Wolting, I. Donaldson, S. Schandorff, J. Shewnarane, M. Vo, J. Taggart, M. Goudreault, B. Muskat, C. Alfarano, D. Dewar, Z. Lin, K. Michalickova, A. R. Willems, H. Sassi, P. A. Nielsen, K. J. Rasmussen, J. R. Andersen, L. E. Johansen, L. H. Hansen, H. Jespersen, A. Podtelejnikov, E. Nielsen, J. Crawford, V. Poulsen, B. D. Sorensen, J. Matthiesen, R. C. Hendrickson, F. Gleeson, T. Pawson, M. F. Moran, D. Durocher, M. Mann, C. W. V. Hogue, D. Figeys, M. Tyers, *Nature* **2002**, *415*, 180 – 183.
[9] G. MacBeath, S. L. Schreiber, *Science* **2000**, *289*, 1760 – 1763.
[10] G. Y. J. Chen, M. Uttamchandani, Q. Zhu, G. Wang, S. Q. Yao, *ChemBiochem* **2003**, *4*, 336 – 339.

[11] G. Y. J. Chen, M. Uttamchandani, R. Y. P. Lue, M. L. Lesaicherre, S. Q. Yao, *Curr. Top. Med. Chem.* **2003**, *3*, 705 – 724.
[12] M. R. Martzen, S. M. McCraith, S. L. Spinelli, F. M. Torres, S. Fields, E. J. Grayhack, E. M. Phizicky, *Science* **1999**, *286*, 1153 – 1155.
[13] N. Jessani, B. F. Cravatt, *Curr. Opin. Chem. Biol.* **2004**, *8*, 54 – 59.
[14] Y. Liu, M. P. Patricelli, B. F. Cravatt, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 14694 – 14699.
[15] G. C. Adam, J. Burbaum, J. W. Kozarich, M. P. Patricelli, B. F. Cravatt, *J. Am. Chem. Soc.* **2004**, *126*, 1363 – 1368.
[16] M. Li, *Nat. Biotechnol.* **2000**, *18*, 1251 – 1256.
[17] J. Hanes, A. Pluckthun, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 4937 – 4942.
[18] S. Zozulya, M. Lioubin, R. J. Hill, C. Abram, M. L. Gishizky, *Nat. Biotechnol.* **1999**, *17*, 1193 – 1198.
[19] V. R. Iyer, C. E. Horak, C. S. Scafe, D. Botstein, M. Snyder, P. O. Brown, *Nature* **2001**, *409*, 533 – 538.
[20] B. van Steensel, S. Henikoff, *Nat. Biotechnol.* **2000**, *18*, 424 – 428.
[21] D. Robyr, Y. Suka, I. Xenarios, S. K. Kurdistani, A. Wang, N. Suka, M. Grunstein, *Cell* **2002**, *109*, 437 – 446.
[22] S. Melkko, J. Scheuermann, C. E. Dumelin, D. Neri, *Nat. Biotechnol.* **2004**, *22*, 568 – 574.
[23] N. Winssinger, J. L. Harris, B. J. Backes, P. G. Schultz, *Angew. Chem.* **2001**, *113*, 3254 – 3258; *Angew. Chem. Int. Ed.* **2001**, *40*, 3152 – 3155.
[24] R. Roberts, J. W. Szostak, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 12297 – 12302.
[25] For synthesis of the probe used in this study, see: Q. Zhu, X. Huang, G. Y. J. Chen, S. Q. Yao, *Tetrahedron Lett.* **2003**, *44*, 2669 – 2672. It should be noted a similar PTP probe was first reported by Lo et al. (L. C. Lo, T. L. Pang, C. H. Kuo, Y. L. Chiang, H. Y. Wang, J. J. Lin, *J. Proteome Res.* **2002**, *1*, 35 – 40).
[26] Q. Zhu, A. Girish, S. Chattopadhaya, S. Q. Yao, *Chem. Commun.* **2004**, 1512 – 1513.
[27] S. Saghatelian, N. Jessani, A. Joseph, M. Humphrey, B. F. Cravatt, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 10000 – 10005.
[28] E. W. S. Chan, S. Chattopadhaya, R. C. Panicker, X. Huang, S. Q. Yao, *J. Am. Chem. Soc.* **2004**, *126*, 14435 – 14446.
[29] L. C. Mattheakis, R. R. Bhatt, W. J. Dower, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 9022 – 9026.
[30] http://www.microarrays.org/protocols.html.

